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Deoxyribonucleic Acid Repair in *Escherichia coli* Mutants Deficient in the 5′→3′ Exonuclease Activity of Deoxyribonucleic Acid Polymerase I and Exonuclease VII

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A series of *Escherichia coli* strains deficient in the 5′→3′ exonuclease activity associated with deoxyribonucleic acid (DNA) polymerase I (exonuclease VI) and exonuclease VII has been constructed. Both of these enzymes are capable of pyrimidine dimer excision in vitro. These strains were examined for conditional lethality, sensitivity to ultraviolet (UV) and X-irradiation, postirradiation DNA degradation, and ability to excise pyrimidine dimers. It was found that strains deficient in both exonuclease VI (*polA*ex$^-$) and exonuclease VII (*xseA*$^-$) are significantly reduced in their ability to survive incubation at elevated temperature (43°C) beyond the reduction previously observed for the *polA*ex single mutants. The UV and X-ray sensitivity of the exonuclease VI-deficient strains was not increased by the addition of the *xseA7* mutation. Mutants deficient in both enzymes are about as efficient as wild-type strains at excising dimers produced by up to 40 J/m^2 UV. At higher doses strains containing only *polA*ex$^-$ mutations show reduced ability to excise dimers; however, the interpretation of dimer excision data at these doses is complicated by extreme postirradiation DNA degradation in these strains. The additional deficiency in the *polA*ex *xseA7* double-mutant strains has no significant effect on either postirradiation DNA degradation or the apparent deficiency in dimer excision at high UV doses observed in *polA*ex single mutants.

Ultraviolet (UV) radiation introduces a variety of lesions in deoxyribonucleic acid (DNA) (21). Principal among these are dimers caused by formation of cyclobutane rings between the 5,6 positions of adjacent pyrimidines. The distortions produced by these dimers must be removed or bypassed if DNA replication is to proceed (22) and the cell is to survive. One mechanism for dealing with pyrimidine dimers is a dark repair process termed excision repair. According to a popular model (13), this repair pathway begins with the recognition of the lesion and the introduction of an incision near the 5′ end of the offending dimer. This is followed by the excision of the dimer together with an adjacent stretch of nucleotides by a 5′→3′ exonuclease. The resulting gap may be filled by DNA resynthesis performed by a DNA polymerase. Repair is completed by ligation of the newly resynthesized DNA with the contiguous parental strand.

At present there are three known 5′→3′ exonucleases in *Escherichia coli* which may be capable of performing the excision step in the model outlined above, since they have been shown to excise pyrimidine dimers in vitro: (i) exonuclease VI, the 5′→3′ exonuclease associated with DNA polymerase I (15); (ii) the 5′→3′ exonuclease associated with DNA polymerase III (18); and (iii) exonuclease VII (4). Mutant strains deficient in two of these exonucleases are presently available. Strains deficient in the 5′→3′ exonuclease activity of DNA polymerase I (*polA*ex$^-$) (12, 14, 16, 23) appear mildly UV-sensitive and somewhat deficient at dimer excision after large UV doses (7, 11, 16), suggesting that exonuclease VI may be involved in the excision repair process. These strains also show temperature-sensitive conditional lethality under certain growth conditions, suggesting that the 5′→3′ exonuclease activity of DNA polymerase I may be essential. The *polA480*ex strain retains about 3% of the 5′→3′ exonuclease activity of DNA polymerase I at 30°C and about 1.5% at 43°C when assayed in vitro, while retaining nearly normal levels of DNA polymerase I activity at both temperatures (16, 23). The *polA546*ex mutants appear to have lower residual exonuclease VI levels than the *polA480*ex mutants (B. Konrad, in press). Strains defi-

cient in DNA polymerase III (*polC*) have been well characterized with respect to the polymerase activity (9), but none has yet been characterized with respect to the 5′→3′ exonuclease activity of the enzyme. Strains deficient in exonuclease VII (*xse*⁻) have recently been isolated (6), and several of these strains (e.g., *xseA7*) have been shown to have less than 1% of the wild-type level of the enzyme. Since exonuclease VII acts exclusively on single-stranded DNA (4,5), it seems ideally suited to the removal of relatively long dimer-containing single-stranded regions which might melt away from the duplex region after the incision event. In this way exonuclease VII might complement the 5′→3′ exonuclease activity of DNA polymerase I in the excision repair pathway, since the latter activity only attacks double-stranded DNA and short, single-stranded regions extending from a duplex region (8, 20).

Because *E. coli* maintains a number of enzymes which can perform similar functions in vitro, excision repair may be able to proceed by alternate enzymatic pathways (13). Thus, deficiencies causing reduced levels of a given repair enzyme might become apparent only when alternate pathways are blocked by the construction of double mutants. We have combined mutations (*polA*ex⁻) causing reduced levels of the 5′→3′ exonuclease activity of DNA polymerase I with a mutation (*xseA7*) causing reduced levels of exonuclease VII to produce a series of *E. coli* strains deficient in one or both enzymes. These strains have been examined for conditional lethality, UV sensitivity, X-ray sensitivity, rates of postirradiation DNA degradation, and ability to excise pyrimidine dimers.

MATERIALS AND METHODS

Bacterial and phage strains. The strains of *E. coli* K-12 used are listed in Table 1. Phages P1CM*clr*100 and P1*kc* were used for generalized transductions (19). Strains KS463, RS5064, and RS5065 were made thymine-requiring by trimethoprim selection as described by Miller (19). These strains were then made *guaA*⁻ by conjugation with strain KLC39 and selection for Thy⁺ colonies. Gua⁺ transductants were isolated from a transduction with P1CM*clr*100 grown on strain KLC7 followed by direct assay for exonuclease VII (6) to produce *xseA7* derivatives of the former strains. These strains were then made thymine-requiring by trimethoprim selection to produce strains KLC124, KLC126, KLC129, KLC130, KLC132, and KLC134. Transduction with phage P1*kc* grown on strain P3478, followed by testing for sensitivity to methyl methane sulfonate, produced strains KLC154 and KLC157 from KLC124 and KLC126, respectively. Bacterial conjugation and P1 transduction were performed as described by Miller (19).

Media and growth conditions. Bacteria were routinely grown with shaking at 30°C unless indicated otherwise. Tryptone broth, L broth, and minimal medium (M9) were prepared as described by Miller (19). Where indicated, L broth was prepared with 0.5% (wt/vol) NaCl instead of 1.0% NaCl. M9 medium was prepared with 0.4% (wt/vol) glucose as carbon source and was routinely supplemented with 0.2% vitamin-free Casamino Acids, 2 μg of thymine per ml, and 40 μg of L-tryptophan per ml. Minimal (M-), trypton (T-), and L-plates were prepared from the medium described above supplemented with 1.5% agar. Tryptone, yeast extract, Casamino

TABLE 1. *Bacterial strains*

Strain	Genotype	Source
KS463	F⁻ *trpA33 rha*⁻	Konrad
RS5064	F⁻ *trpA33 polA480*ex[a]	Konrad
RS5065	F⁻ *trpA33 polA546*ex[a]	Konrad
P3478	F⁻ *thyA36 polA1 dra-2* λ⁻	*E. coli* Genetic Stock Center Strain 4303
KLC7	F⁻ *thr-1 leu-6 his-1 argH1 lys-25 lacY1 malA1 xyl-7 ara-13 mtl-2 gal-6 purE43 tonA2 thi-1 str-9* λ⁻ *xseA7*	Chase
KLC39	Hfr KL16 serA ◀ lipA *guaA21 his*⁻ *str*ʳ	Chase
KLC124	F⁻ *trpA33 rha*⁻ *thy*⁻	This paper
KLC126	F⁻ *trpA33 rha*⁻ *thy*⁻ *xseA7*	This paper
KLC129	F⁻ *trpA33 thy*⁻ *polA480*ex *xseA7*	This paper
KLC130	F⁻ *trpA33 thy*⁻ *polA546*ex	This paper
KLC132	F⁻ *trpA33 thy*⁻ *polA546*ex *xseA7*	This paper
KLC134	F⁻ *trpA33 thy*⁻ *polA480*ex	This paper
KLC154	F⁻ *trpA33 thy*⁻ *polA1*	This paper
KLC157	F⁻ *trpA33 thy*⁻ *polA1 xseA7*	This paper

[a] Following the suggestion of the editors of *J. Bacteriol.* and with the agreement of I. R. Lehman and B. Konrad, the previous designations *polAex1* and *polAex2* have now been changed to *polA480*ex and *polA546*ex, respectively.

Acids, and agar were purchased from Difco. For the temperature-shift experiments in Table 2, the plating medium at 30°C was always the same as the corresponding one at higher temperature. Also, in these experiments the plates used for incubation at the elevated temperature were warmed to that temperature for several hours before the bacteria were spread. The plates were then incubated overnight at 30, 43, or 44.5°C.

UV irradiation. Before irradiation, exponentially growing cultures at a density of 2×10^8 to 5×10^8 cells/ml (as estimated by absorbance at 590 nm) were diluted with M9 salts (M9 medium without glucose or other additives), collected by centrifugation, and washed twice in M9 salts. The cells were resuspended in M9 salts at 10^8 to 2×10^8 cells/ml and irradiated at room temperature, 3 ml at a time in 7.5-cm-diameter watch glasses with constant stirring. A pair of germicidal lamps with an incident dose rate of 1.04 J/m² per s (0.89 J/m² per s in some experiments) provided the UV source. Irradiation and all subsequent manipulations were carried out in the dark or under yellow light. For measuring UV survival, irradiated cells were diluted in 0.85% NaCl before being spread on appropriate plates.

X-irradiation. For measurements of X-ray survival, bacteria growing exponentially in tryptone broth were diluted and washed in M9 salts. Cells at a density of about 5×10^8 cells/ml were irradiated at room temperature, 4 ml at a time in 5-cm-diameter plastic petri dishes with a 1-mm plastic cap. Irradiation was done with a General Electric Maxitron 250 X-ray machine equipped with a Be window X-ray tube and 3-mm Al filter. The exposure rate was 1 Krad/min. Immediately after irradiation, the cells were diluted in tryptone broth and spread in duplicate on T-plates. The plates were incubated at 32°C.

DNA degradation. Overnight cultures of bacteria were diluted 1:100 in M9 medium containing 1 μCi per 2 μg per ml [*methyl*-³H]thymine (purchased from Schwarz/Mann). The cells were grown with shaking at 32°C to a density of about 4×10^8 cells/ml before being harvested, washed, and UV-irradiated as described above. Duplicate 0.1-ml samples were withdrawn and the bacteria were precipitated with ice-cold 5% (wt/vol) Cl_3CCOOH. Amounts of 2 to 4 ml of irradiated cells in a 50-ml flask were shifted to the elevated temperature (as indicated) by placing the flask in a gyratory shaking water bath and allowing 5 min before the addition of glucose, thymine, Casamino Acids, and tryptophan. At intervals, 0.1-ml samples were withdrawn, precipitated with Cl_3CCOOH, and collected on Reeve-Angel glass-fiber filters. Radioactivity was determined by use of a scintillation fluid made from 3 g of Packard Permablend per liter of toluene.

Dimer excision. Their capacity for excising thymine-containing dimers was determined by growing bacteria in M9 medium as described above with [³H]thymine at 25 μCi per 2 μg per ml. Cells were harvested, irradiated as described above, and warmed to 42°C for 5 min before the addition of nutrients. Samples of 1 ml were withdrawn at the indicated times and quickly chilled to 0°C. The samples were collected by centrifugation and frozen at −20°C. The samples were then thawed and immediately handled in one of two ways. (A) The samples were resuspended in 0.5 ml of 0.01 M NaCl, 0.001 M ethylenediaminetetraacetate (EDTA), 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8.0. A 2-ml amount of ice-cold 10% Cl_3CCOOH was added, and the precipitate was collected by centrifugation. Then 0.1 ml of salmon sperm DNA (0.5 mg/ml) was added, followed by 2 ml of ice-cold 10% Cl_3CCOOH. The precipitate was collected by centrifugation and washed once with ice-cold ethanol. After drying, the samples were digested with formic acid, and the content of thymine-containing dimers was determined by two-dimensional paper chromatography as described by Carrier and Setlow (3). (B) In some cases, resolution of dimers seemed to be improved by deproteinizing the sample. In these experiments the samples were resuspended in 0.5 ml of 0.01 M NaCl, 0.02 M EDTA, 0.01 M Tris-hydrochloride, pH 8.0, with 0.5% (wt/vol) sodium dodecyl sulfate. After 20 min of incubation at 37°C, 1 ml of phenol saturated with 0.05 M sodium phosphate (pH 7.4) was added to the lysate, and the mixture was blended vigorously in a Vortex mixer for 2 min. After centrifugation, the aqueous layer was withdrawn, 0.1 ml of salmon sperm DNA (0.5 mg/ml) was added, and the sample was chilled to 0°C. A 1-ml amount of ice-cold 20% Cl_3CCOOH was added and the precipitate was collected. After washing once with 10% Cl_3CCOOH and once with ethanol, the sample was digested with formic acid and the thymine dimer content was determined (3).

RESULTS

Temperature sensitivity of *polA*ex⁻, *xseA*⁻, and *polA*ex⁻ *xseA*⁻ strains. Under certain growth conditions, *polA480*ex and *polA546*ex mutants show reduced viability at elevated temperatures (16). To see whether the *xseA7* mutation would affect viability when combined with *polA*ex mutations, we examined strains carrying combinations of these mutations for ability to form colonies at elevated (43°C) temperature. Since unusually high temperatures (44.5°C) appear to reduce further the ability of *polA480*ex mutants to form colonies (B. Konrad, personal communication), the strains were compared for survival at 30, 43, and 44.5°C. Also, since high NaCl concentrations affect the ability of *polA480*ex mutants to survive at elevated temperatures (P. Cooper, Mol. Gen. Genet., in press), L-plates containing low salt (0.5% NaCl) or high salt (2.0% NaCl) were used. From the data summarized in Table 2, it is apparent that the *xseA7* mutant and wild-type cells are equally able to survive incubation at 43 or 44.5°C under the growth conditions tested. Although the survival of the *polA546*ex mutant was somewhat affected by incubation at either 43 or 44.5°C on low-salt L-plates, this mutant showed greatly reduced

TABLE 2. *Percent survival at elevated temperature relative to survival at 30°C*

Strain	Genotype	L broth				M9 broth	
		0.5% NaCl		1.0 % NaCl		44.5°C	43°C
		43°C	44.5°C	43°C	44.5°C		
KLC124	Wild type	109	109	89	103	298	
KLC126	*xseA7*	118	111	96	101	325	
KLC130	*polA546*ex	62	53	1.1	0.13	0.38	0.55
KLC132	*polA546*ex *xseA7*	0.13	0.05	0.06	0.017	0.024	
KLC154	*polA1*	124	152	3	3	104	106
KLC157	*polA1 xseA7*	88	102	3	3	7.3	1.7

survival when a higher NaCl concentration was used. Under identical conditions, the *polA546*ex *xseA7* strain showed lower survival than the *polA546*ex strain. Reduced survival was also observed when *xseA7* was combined with *polA546*ex and growth was attempted on minimal plates. Similar results were obtained with a *polA480*ex *xseA7* strain (data not shown).

Strains containing the *polA1* mutation alone (which results in reduced levels of DNA polymerase I activity, but nearly normal levels of the 5′→3′ exonuclease activity of DNA polymerase I [17]) and in combination with the *xseA7* mutation were also tested for ability to survive elevated temperature (Table 2). The *polA1* strain, like the *polA*ex mutants, showed sensitivity to high NaCl concentrations when plated on rich media. However, in contrast to results with *polA*ex mutants, the addition of the *xseA7* mutation to the *polA1* strain had little effect when survival was assayed on L broth plates. The *xseA7* mutation did reduce the ability of strains with a *polA1* mutation to survive elevated temperature when incubated on minimal plates.

UV and X-ray sensitivity of *polA* ex⁻, *xseA7*, and *polA* ex *xseA7* strains. Since both exonucleases VI and VII have been implicated in excision repair of UV-irradiated DNA (1, 5, 13, 15), it was of interest to test the ability of strains with deficiencies in one or both of these enzymes to survive UV irradiation. Because of the sensitivity to growth conditions seen in Table 2, UV survival was tested on both rich and minimal media. Figure 1 shows the percent survival of each strain as a function of UV dose. Both *polA* ex mutations caused increased sensitivity to UV. Although the *xseA7* mutant was only slightly more UV-sensitive than the wild-type, the combination of *xseA7* with either *polA*ex mutation showed increased UV sensitivity when the cells were incubated on rich

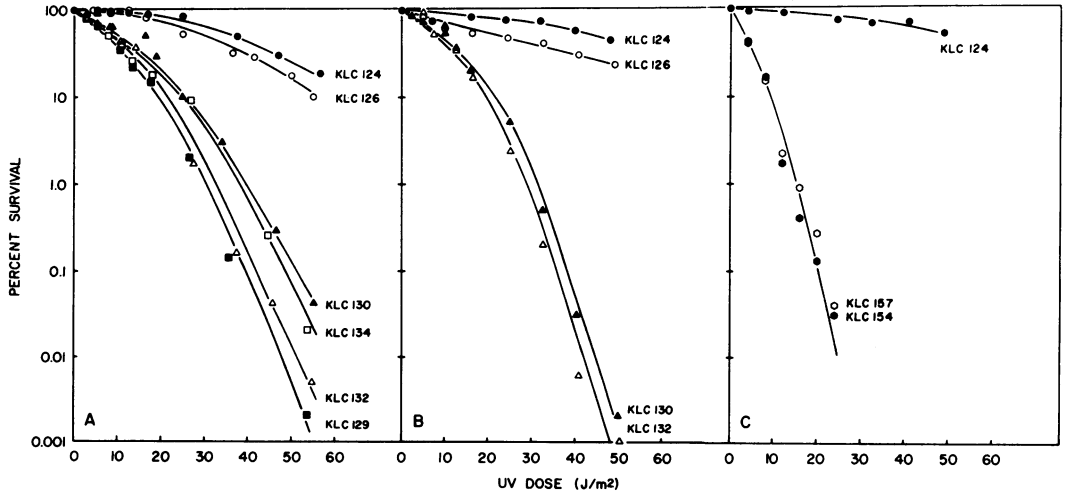

FIG. 1. *UV survival of polAex⁻, xseA7, and polAex⁻ xseA7 strains. Cultures growing exponentially in L broth or M9 minimal medium were irradiated with various doses of UV as described in Materials and Methods and plated on L broth (A) or M9 minimal (B and C) agar plates. Plates were incubated at 30°C. Symbols: ●, strain KLC124 (wild type); ○, strain KLC126 (xseA7); ■, strain KLC129 (polA480ex xseA7); ▲, strain KLC130 (polA546ex); △, strain KLC132 (polA546ex xseA7); □, strain KLC134 (polA480ex); ⬢, strain KLC154 (polA1); ⬡, strain KLC157 (polA1 xseA7).*

media (Fig. 1A). However, when the cells were grown on minimal plates, there was little difference in UV sensitivity between *polA546*ex and *polA546*ex *xseA7* mutants (Fig. 1B). Also, the combination of *xseA7* with *polA1* caused no increase in UV sensitivity when grown on minimal media (Fig. 1C). Thus, although the growth conditions influence the UV sensitivity of these strains, the *xseA7* mutation caused only a small (perhaps negligible) effect on UV survival when growth was attempted on minimal media. When UV sensitivity was tested with incubation at 44.5°C, the *xseA7* mutation caused slightly increased UV sensitivity when combined with either *polA480*ex or *polA546*ex (data not shown). However, because the unirradiated strains show reduced viability at high temperature (Table 2), UV sensitivity data at this temperature are difficult to interpret.

Similar experiments were performed to test the ability of mutants deficient in either or both exonucleases VI and VII to survive damage caused by X-irradiation in air (data not shown). Although the exonuclease VI-deficient strains are somewhat X-ray-sensitive, the *xseA7* mutation causes no X-ray sensitivity by itself, nor does it reduce the survival of the *polA*ex mutants. Thus, these data suggest that exonuclease VII does not play a key role in the repair of damage caused by ionizing radiation.

Pyrimidine dimer excision in *polA*ex⁻, *xseA7*, and *polA*ex⁻ *xseA7* strains. In vitro studies suggest that both exonucleases VI and VII may act to excise pyrimidine dimers once incision takes place. Therefore, it was important to see whether in vivo dimer excision deficiency is associated with the *polA* ex mutations. Although *xseA7* mutants are not UV-sensitive (Fig. 1), it is possible that dimer excision deficiency in these strains might be masked by other dark repair processes (13, 24) which might contribute to the overall survival of UV-irradiated cells. Therefore, a study of dimer excision in *polA*ex and *xseA7* mutant strains was undertaken. Cultures labeled with [^{3}H]-thymine were irradiated and warmed to 42°C, and samples were examined for thymine-containing dimers. Figure 2 shows the percent dimers remaining as a function of incubation time. Comparison of mutant strains with wild type shows that the *polA*ex and *xseA7* mutations, either alone or in combination, have little effect on the extent of in vivo dimer excision. However, since most of the dimers are removed by 15 min in all the strains tested, these data say little about the kinetics of excision immediately subsequent to irradiation.

The results in Fig. 2 were obtained with a dose of UV which allows survival of about 10% of the most sensitive cells (Fig. 1). To see if dimer excision deficiencies could be detected at higher doses, we performed the experiment shown in Fig. 3. Cultures were divided and irradiated with various doses of UV. One-half of each portion of irradiated cells was kept at 0°C; the remainder was warmed to 42°C and then incubated with nutrients for another 30 min. The samples were then examined for thymine dimer content. Figures 3A and 3B show that both the wild type and the *xseA7* mutant can remove almost all the thymine dimers introduced by UV doses up to 100 J/m^2. The *polA546*ex mutant, shown in Fig. 3C, can remove most of the dimers that accumulate up to

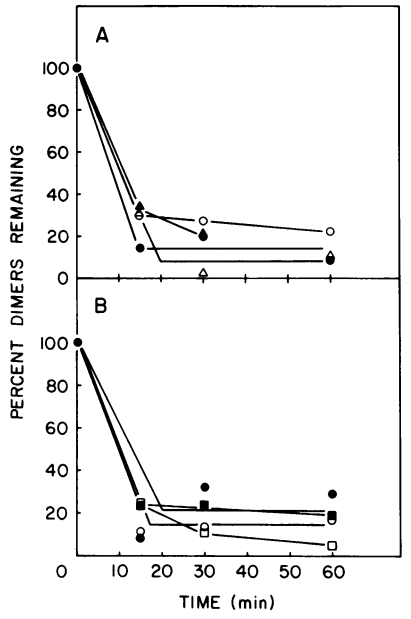

FIG. 2. *Pyrimidine dimer excision of polAex⁻, xseA7, and polAex⁻ xseA7 strains. Exponentially growing cultures were labeled with [^{3}H]thymine in M9 minimal medium as described in Materials and Methods. The cells were washed free from nutrients and irradiated with 20 J/m^2 UV. Samples (1 ml) were withdrawn immediately before and after irradiation. The cells were warmed to 42°C for 5 min before the addition of nutrients. Samples were withdrawn at the indicated times after addition of nutrients. (A) The samples were examined directly for pyrimidine dimers (scheme A in Materials and Methods). (B) The samples were lysed and the DNA was extracted with phenol before being examined for content of pyrimidine dimers (scheme B in Materials and Methods). The background of ^{3}H found in unirradiated controls has not been subtracted. This background value corresponds to about 11% in A and 7% in B. The symbols are the same as those for Fig. 1.*

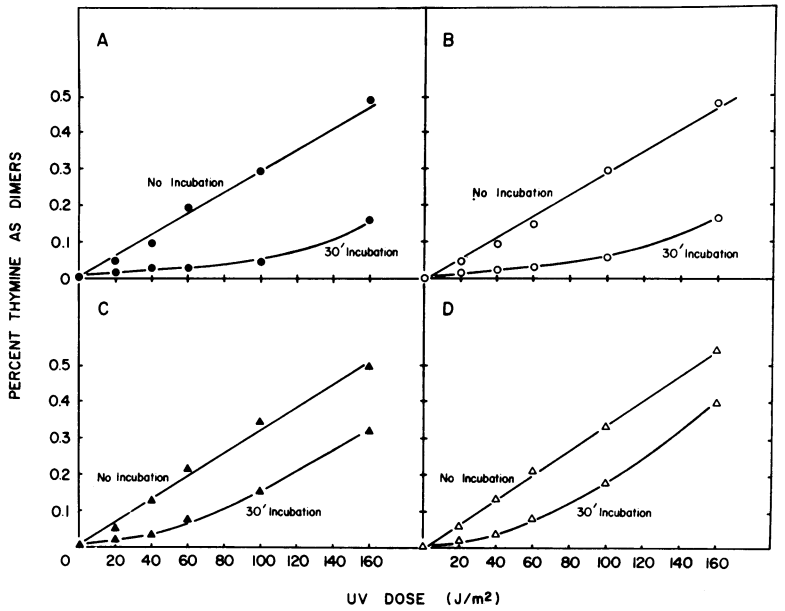

FIG. 3. *Dimer excision as a function of UV dose. Exponentially growing [³H]thymine-labeled cultures were washed and irradiated as described in Materials and Methods. One-half of each sample was kept as a control at 0°C; the other half was warmed to 42°C for 5.0 min, and then nutrients were added. After 30 min of incubation at 42°C, the samples were chilled, and the amounts of thymine dimers were determined according to scheme A in Materials and Methods. (A) Strain KLC124 (wild type); (B) strain KLC126 (xseA7); (C) strain KLC130 (polA546ex); (D) strain KLC132 (polA546ex xseA7).*

a dose of 40 J/m². However, this mutant can excise only a fraction of the thymine dimers resulting from higher UV doses. The double mutant *polA546*ex *xseA7*, shown in Fig. 3D, gave results identical to those found with the *polA546*ex strain. These data, together with similar results (not shown) obtained with a *polA480*ex *xseA7* double mutant, indicate that an additional deficiency in exonuclease VII does not stress the repair capacity of *E. coli* to the point where further dimer excision deficiencies become apparent.

Since Table 2 shows that the *polA*ex mutants are sensitive to incubation at unusually high temperatures (44.5°C), part of the experiment described above was repeated at this elevated temperature. The results of this experiment (data not shown) were identical with results obtained at lower (42°C) temperatures.

Postirradiation DNA degradation in *polA*ex⁻, *xseA*⁻, and *polA*ex⁻ *xseA*⁻ strains. Aberrant processes, such as postirradiation DNA degradation, would almost surely affect dimer excision and cause reduced survival. To estimate the extent of DNA degradation after low and high doses of UV, we performed the experiment shown in Fig. 4. Cultures labeled with [³H]thymine were irradiated with 20 or 100 J/m² before being warmed to a restrictive temperature. At various times after the addition of nutrients, samples were withdrawn and precipitated with Cl_3CCOOH, and acid-insoluble radioactivity was determined. Figure 4A shows that after irradiation with 20 J/m² the *polA*ex and *xseA7* mutants degrade their DNA to about the same extent as the wild type. However, after 100 J/m², strains with *polA*ex mutations degrade their DNA more rapidly than wild-type or *xseA7* strains (Fig. 4B). Similar results were obtained when the incubation was carried out at 44.5°C (Fig. 4C). Thus, after high doses of UV as much as 50% of the DNA may be degraded in 30 min of incubation. This DNA degradation may be partially responsible for the UV sensitivity and apparent dimer excision deficiency we observed.

DISCUSSION

The construction of a series of strains containing the *polA*ex (deficient in exonuclease VI) and *xseA* (deficient in exonuclease VII) mutations has allowed us to begin an analysis of the 5′→3′ exonucleases thought to be involved in excision repair. The effects of various media and temperatures on the viability of strains deficient in exonucleases VI and VII have demonstrated that survival of *polA*ex⁻ strains is significantly affected by the concentration of

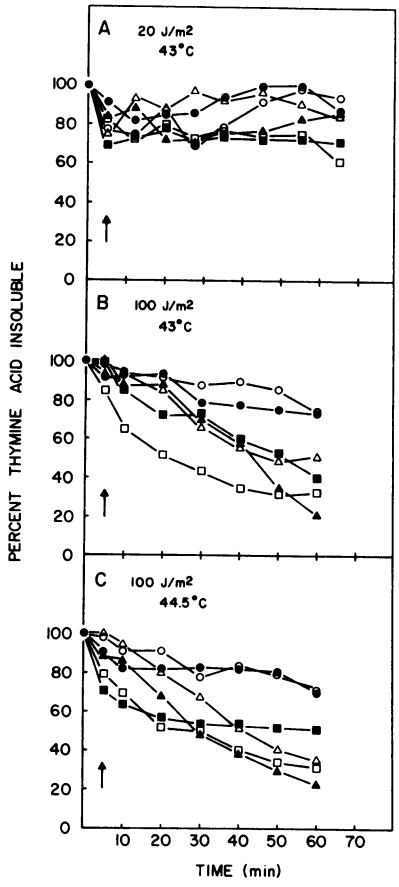

FIG. 4. *Postirradiation DNA degradation. Exponentially growing cultures labeled with [^{3}H]thymine were washed and irradiated with 20 J/m^2 (A) or 100 J/m^2 (B and C). The cultures were warmed to 43°C (A and B) or 44.5°C (C) before addition of nutrients (shown by the arrow). At the indicated times, samples were withdrawn, chilled, and precipitated with ice-cold Cl_3CCOOH. Percent acid-precipitable radioactivity is plotted as a function of incubation time. The symbols are the same as those for Fig. 1.*

NaCl when grown in rich media at 43°C (Table 2), with low NaCl concentrations favoring survival. Although no such effects were observed with the *xseA7* strain, the *polA*ex$^-$ *xseA7* double-mutant strain showed reduced survival under all conditions of growth at elevated temperatures compared with 30°C, and the effect was most dramatic on rich plates containing high concentrations of NaCl. The interpretation of this result is unclear, but our observation demonstrates that the loss of two specific 5′→3′ exonuclease activities is very damaging to the cell. This may indicate that exonucleases VI and VII together function in some essential DNA metabolic process (possibly one of them serving in a back-up capacity) and that the further reduction in these activities caused by elevated temperatures stresses the system to the point that it can no longer function adequately.

The survival of these strains after exposure to UV irradiation demonstrates that strains carrying *polA*ex mutations are not as sensitive as a *polA1* strain. In addition, the *xseA7* mutation does not appear to increase significantly the UV sensitivity of strains carrying this mutation over wild-type strains. However, strains carrying both *polA*ex and *xseA7* mutations are significantly more UV-sensitive than *polA*ex single mutants when grown in rich media (Fig. 1). When a similar experiment was performed with cells grown in minimal medium, the double mutants appeared to be no more sensitive than the *polA*ex$^-$ single-mutant strains. In terms of the excision repair functions of exonucleases VI and VII, these media effects are difficult to interpret; however, again, it is clear that *polA*ex$^-$ *xseA7* strains show defects greater than those of either single mutant under certain growth conditions.

In vitro studies suggest that the 5′→3′ exonucleases associated with DNA polymerases I and III and the 5′→3′ activity of exonuclease VII may all function in excision repair, since all are able to excise pyrimidine dimers in vitro (4, 15, 18). However, our results (Fig. 2 and 3) show that at UV doses up to 40 J/m^2 even the *polA*ex *xseA7* double mutants excise dimers to about the same extent as wild type. The *polA*ex$^-$ strains appear to be less efficient than wild type at dealing with dimers produced by higher doses of UV. However, the postirradiation DNA degradation shown in Fig. 4 makes interpretation of the dimer excision data in Fig. 3 somewhat difficult. Identification of the nucleases responsible for this degradation, together with studies of the in vitro properties of these enzymes, may provide evidence as to whether dimer-containing DNA is degraded to the same extent as DNA without these lesions. Also, construction of mutants deficient in certain nucleases may prove helpful. Since exonuclease V (the RecB,C enzyme) is known to be involved in some types of postirradiation DNA degradation (2), the construction of *polA*ex *recB* ts mutants may be helpful in controlling DNA degradation. If we assume that DNA is degraded at the same rate, irrespective of whether or not it contains pyrimidine dimers, the results shown in Fig. 3 suggest that the *polA*ex mutants have a limited capacity for dimer excision and that damage accumulated during exposure to high (>40 J/m^2) UV doses cannot be corrected. Previous work showing a reduced rate and extent of dimer excision in

exonuclease VI-deficient strains (7, 11) subjected to high doses of UV tends to support this argument. The residual levels of exonuclease VI activity present in the *polA*ex mutants might be sufficient to repair damage accumulated during exposure to low doses of UV, but insufficient to repair all damage at high doses of UV. Nonetheless, if exonuclease VI is the only enzyme responsible for in vivo dimer excision, it is surprising that experiments like the one in Fig. 2 show no difference in the extent of dimer excision when the *polA*ex mutants are compared with wild type. The *xseA7* mutation, either alone or in combination with the *polA*ex mutations, had no apparent effect on dimer excision, even at very high UV doses. Although arguments concerning residual levels of exonuclease VII activity can be made, this result suggests that, even if exonuclease VII functions as an auxiliary repair enzyme, other enzymes also function in this capacity.

The results presented here suggest that a deficiency in two specific 5′→3′ exonuclease activities, both of which excise pyrimidine dimers in vitro, does not seriously affect in vivo dimer excision. Although the question of whether or not either exonuclease VI or exonuclease VII is essential in the excision repair pathway cannot be conclusively answered from this work (since all of the strains used contain residual amounts of both enzymes), these results do strongly suggest that neither of these enzymes is solely responsible for in vivo excision. The results also suggest that excision can occur at nearly normal rates, at least at low doses of UV, when deficiencies occur in either or both of these enzymes. It will be necessary in future work to examine the role of the 5′→3′ exonuclease activity of DNA polymerase III and possibly to use strains lacking several 5′→3′ exonucleases to search for new enzymatic activities and eventually to characterize the entire excision repair pathway. In this regard it may be productive to examine *mfd* mutant strains, since they exhibit reduced rates of dimer excision in vitro (10), but have normal levels of exonuclease VII activity (J. W. Chase, unpublished data).

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